ARRANGEMENT FOR OPTIMIZING THE PULSE SHAPE IN A LASER SCANNING MICROSCOPE

BACKGROUND OF THE INVENTION

Field of the Invention

At present, nonlinear contrasts such as two-photon absorption or second harmonic generation (SHG) are used to an increasing extent in microscopy, e.g., for examination of biological preparations. It is advantageous to use short pulse lasers to provide the energy needed to excite nonlinear effects. In this connection, the peak pulse power should be as high as possible and the pulse length at the location of the specimen should accordingly be as small as possible to prevent damage to the preparation simultaneously. Short pulse lasers supply light pulses, for example, of several 10fs at a repetition rate of several 10 MHz. Accordingly, they have the advantage that they emit extremely high peak pulse energies accompanied at the same time by low average output.

It is disadvantageous that the short pulses on the path through the microscope to the specimen change due to the group velocity dispersion (GVD) - usually, they become longer.

Description of the Related Art

In order to compensate for pulse lengthening, corresponding changes (prechirp devices) have been suggested (DE 19622353).

Further, adaptive optics have been provided in DE 19733193.

The described devices are suitable for compensation of second-order dispersion.

However, higher-order dispersions which cannot be determined beforehand must be taken into account, e.g., in biological preparations. Further, higher-order dispersions occur in the optical components in a microscope. Therefore, it is not possible to create optimum conditions for the excitation of nonlinear contrasts by conventional techniques.

In conventional fluorescence microscopy, different dyes are used for specific tagging of biological preparations. These dyes are subsequently excited by different light wavelengths. In preparations of this kind, simultaneous excitation of the various dyes is usually carried out using multiphoton excitation. On the one hand, this is advantageous because only one light wavelength is needed for excitation. On the other hand, it is disadvantageous when the emission wavelength

bands of the individual dyes overlap because the dyes can then no longer be spectrally separated. ~~It is the object of the invention to overcome the described disadvantages~~.

~~Arrangement:~~

A block diagram of the preferred construction is shown in Fig. 1.

The light pulses proceed from the short pulse laser KL to the pulse shaper PF. The latter is shown schematically in Fig. 2a. In the pulse shaper PF, the incident beam (beam in) is spatially split into the spectral components of the light pulses in a first dispersive element (1) comprising, e.g., a grating or prisms. A Fourier plane is then generated by means of an achromatically corrected lens or lens group L1 (Fig. 2).

This plane (focal plane) is characterized in that the individual spectral components of the light pulses are spatially separated. Considered mathematically, the transformation into this plane corresponds to a Fourier transform. In this plane, a spatial light modulator (2) (SLM) is used in transmission. Generally, it comprises a matrix of nematic liquid crystals (e.g., SLM-S160/h, Jenoptik LOS) in helical or parallel arrangement. The transmission and phase displacement of the corresponding spectral components can be adjusted by a corresponding electronic arrangement of the individual points of the matrix. The spatial separation of the spectral components of the light pulses is then canceled by a second identical lens L2 and a second dispersive element (3) (beam out) identical to the first dispersive element. This process corresponds to the inverse transform in the time domain. Therefore, the time behavior of the light pulses can be controlled by means of phase modulation or amplitude modulation. The arrangement of 2 gratings and 2 lenses is known from the literature as a 4f system.

A simplified arrangement for the pulse shaper is shown in Fig. 2b. In this case, a mirror S is arranged right after the modulator (2) so that the beam runs back into itself with a small vertical offset or at a small angle. First, this arrangement makes do with few optical components; second, the light pulses traverse the modulator (2) twice, so that the magnitude of the phase/amplitude modulation is doubled.

Fig. 3 shows schematically the dispersive splitting and combination of a red component r and a blue component b passing the manipulator 2 and the wavelength shape along a direction X to the manipulator 2.

Since the time behavior can be changed in the pulse shaper, the light pulses pass via corresponding optical components via the microscope M and the objective V into the specimen P. A nonlinear effect is excited in the specimen P because of the sharp focussing through the objective and the high peak pulse power of the light pulses. This nonlinear effect is recorded by the detector (4). Therefore, a corresponding measurement signal is available that can be optimized by electronically controlling the pulse shaper by means of regulation R.

The operation of the regulation will be described by way of example of generation of a two-photon fluorescence signal.

The two-photon fluorescence signal (S) can be described as follows:

$S \propto \frac{P_{avg}^2}{\tau^2 \cdot A^2}$, where P_{avg} is the average output and τ is the pulse length of the light pulses at the location of the specimen. A stands for the beam cross section at the location of the specimen interaction.

It can be seen from the above equation that the two-photon fluorescence signal increases as the pulse length and beam cross section decrease and as average output increases. In a microscope, the pulse length is influenced, i.e., usually lengthened, by the following factors:

- the glass materials from which the optical elements in the microscope are made; compensation can be carried out in a stationary manner;
- the specimen in itself; in this case, the lengthening of the pulse depends upon the depth of penetration into the specimen; further, the pulse widening is generated by higher-order dispersions; therefore, compensation must be carried out for every spectral component individually and in real time;
- change in wavelength;
- change in average output.

The pulse shaper PF, and accordingly the time behavior of the light pulses, is therefore adjusted by regulation in real time depending on the above-mentioned variables, wherein the two-photon fluorescence signal functions as a measured quantity. Essentially the pulse length and the average output at the location of specimen interaction are optimized by the pulse shaper.

Further, the interaction cross sections of the utilized dyes are dependent on the time behavior of the light pulses. Accordingly, it is possible to optimize the fluorescence signal for individual dyes, wherein the fluorescence of other dyes is simultaneously suppressed. This is known in the literature as coherent control. Thus, by feeding back the measured quantity (in this case, the two-photon fluorescence signal), it is possible to adjust the time behavior of the light pulses by phase modulation or amplitude modulation in such a way that the corresponding measured quantity is optimized.